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COMMENTARY

MOLECULAR BASIS FOR BINDING PROMISCUITY OF ANTAGONIST DRUGS

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The non-specificity of drugs is a generally acknowledged truism. The inevitable side-effects associated with drug therapy reflect in part an impact on cell components extraneous to the principal target, usually a functional protein, against which the chemical agent is directed. Membrane receptors, enzymes, transporters and channels are being identified and characterized, and novel drugs synthesized, in ever-increasing numbers. Accordingly, interactions between a given drug class and multiple protein species are being reported with increasing frequency. Also, technical advances, such as radioligand binding assays and single channel recording, permit rapid and quantitative assessment of drug–protein interactions. In many instances, multiple functional proteins are perturbed by a single compound and at the same concentration. Compounds that antagonize endogenous ligands constitute a major proportion of clinical agents. Clearly, indiscriminate interactions between an antagonist drug and a variety of membrane acceptors may result in multifarious pharmacodynamics if the drug shows similar affinities for several functional proteins. Examination of the chemical structures of commonly used drugs and of functional proteins reveals commonalities that suggest a basis for overlapping specificities among pharmacological classes of drugs, on the one hand, and among the ligand-accommodating proteins, on the other hand.

Ligand-binding domains on functional membrane proteins: Variations on a structural theme

Membrane proteins in general are globular macromolecules, and analyses of tertiary structures define similarities among the proteins in a particular membrane. Even more extensive similarities are indicated among proteins with α-helical, β-configuration, or mixtures of these two types of chain folding. Limited diversity in topological and three-dimensional features of proteins in general is expressed in similarities in the sizes of domains (folding units) and derives from the fact that energetics favor particular types of folding [see references in Ref. 1]. Thus, functional membrane proteins share, both within a given family and between families, common structural features that provide for macromolecules with more hydrophilic amino and carboxy termini exposed to one or both extracellular and cytoplasmic environments and connected by hydrophobic membrane-spanning domains. This structural theme occurs among the G-protein-linked receptors, ATP-driven cation pumps, ion channels, adenylyl cyclase, the γ-aminobutyric acid (GABA) transporter, and P450 enzymes, among others.

A central hydrophobic core, dominated by lipophilic amino acid side chains, constitutes another common, or even universal, feature of globular proteins. Preservation of amino acid sequences to select for a stable, minimum energy conformation may form the basis for similarities in the generation of hydrophobic domains, including the dimensions of crypts within the protein core. Specificity of a protein crypt for certain endogenous ligands may be established by virtue of amino acid residues lining the crypt and comprising hydrophobic, polar and charged residues. Crevices within the hydrophobic core of the protein constitute the ligand binding domain on functional proteins as diverse as the mammalian β-adrenergic receptor [2] and bacterial reductases, kinases, dehydrogenases, and carbohydrate-binding proteins [3]. A carboxylate moiety commonly forms a complex with the charged nitrogen of the ligand in the case of G-protein-linked receptors, HIV-1 protease, cellobiohydrolase, muscarinic receptor, and ion channels, among others.

Endogenous ligands (e.g. bioamines, steroids, amino acid transmitters, and prostaglandins and other eicosanoids), their agonist and antagonist analogues, and drugs in general, including general anesthetics, are restricted to molecular weights of less than 400, the majority less than 300. Furthermore, receptor recognition domains on relatively large hormonal peptides and proteins are restricted to a few amino acid residues, compatible with the thesis that biologically relevant acceptor sites for endogenous mediators in general are of similar restricted dimensions [see Ref. 1]. For example, a depth of 6 Å was estimated for the pocket of dipeptidyl peptidase that binds side chains of peptide substrates [4]. The hydroxylating enzyme, cytochrome P448, accommodates substrate molecules that are planar and with high area/depth ratios (e.g. chlorpromazine: $11 \times 6 \times 4$ Å) but not by bulky molecules with low area/depth radios (e.g. diphenylhydantoin $11 \times 7 \times 9$ Å) [5]. The latter substrate is accommodated by other P450 isozymes. The nicotinic, hydrophobic receptor channel has its widest diameter at the opening (25–30 Å) but accommodates agonist and antagonist ligands

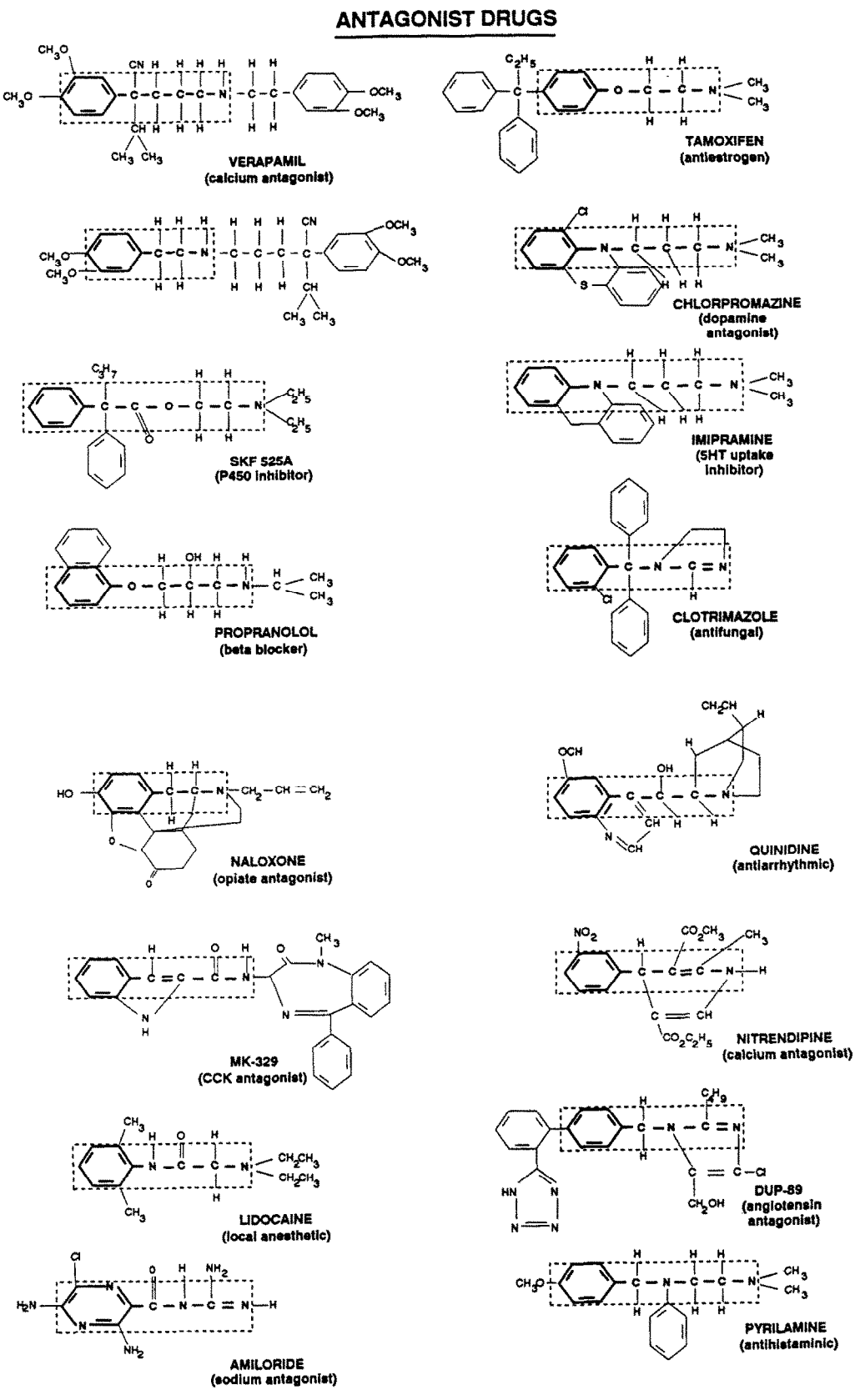

Fig. 1. Structures of selected antagonist drugs. The common pharmacophore is within the rectangle formed by the dotted line. For each drug, the generally accepted pharmacological designation is shown in parentheses.

within narrower portions of the channel [6]. A benzodiazepine that potently inhibits (IC_{50}: 0.08 nM) the binding of cholecystokinin (CCK) and a cyclic hexapeptide derivative of somatostatin both occupy a space within the target with dimensions of 10×15 Å [7].

Furthermore, in keeping with a general theme of structural commonality among proteins, sulfhydryl and/or disulfide groups are implicated in binding of ligands to recognition sites of receptors for histamine, insulin, glucocorticoids, dopamine, angiotensin II, β-adrenergics, α-adrenergics, neurotensin, opiates, acetylcholine, calcium channel antagonists, and glutamate, to amino acid and serotonin transporters, and to the substrate binding pocket of P450 enzymes.

Antagonists for drugs and hormones: Variations on a structural theme

An obvious, common, two-dimensional feature among drug agonists and antagonists comprises an aromatic ring at one end of the molecule and, at the other, an ionizable, usually secondary or tertiary, nitrogen (Fig. 1). This structural theme has been noted for CNS drugs by Andrews and Lloyd [8] who point out that the aromatic moieties are all capable of forming van der Waals' interactions with a planar receptor surface. The common nitrogen, whether charged or not, is in each case able to donate a proton. The aromatic (usually phenyl) ring, free or fused, is connected by a 2–5 atom chain to the nitrogen atom, the latter as an amine, amide, or imine. This pharmacophore (Fig. 1) would seem to be an even more ubiquitous drug commonality than proposed by Andrews and Lloyd.

The substituents projecting from the common pharmacophore determine specificity of a given drug for a particular protein. In this regard, thirty-three opiate agonist and antagonist drugs are characterized by a tertiary nitrogen/benzene ring pharmacophore, plus additional features required for binding to both the agonist and antagonist configurations of the mu receptor; however, certain other molecular features that differ between agonists and antagonists interact with respective, complementary subsites within the receptor binding pocket [9]. A conformation–activity relationship among most of the known 5-hydroxytryptamine (S_3) receptor antagonists led to a model wherein the common pharmacophore is characterized by a carbonyl group, coplanar to an aromatic ring and situated between the basic nitrogen and the aromatic moiety [10].

In Table 1 are listed interactions of each of several drugs, representing different pharmacological classes, with various species of functional proteins. The calcium antagonists and local anesthetics were combined, because of the paucity of reports for individual compounds. Most of the reported values are derived from binding data, but others are from functional experiments, such as those in which enzyme activity and ion flux were measured. This catalog of interactions between drug and functional proteins is, by no means, to be considered exhaustive. Only a few compounds have been selected to represent classes of drugs generally believed to act, primarily, at a single locus, e.g. antiadrenergic, antihistaminic or calcium antagonist drugs. Almost certainly, some published reports of promiscuity for drugs listed in the table have been overlooked, and for that apology is made. It is likely that future experimentation will reveal many more interactions.

The compounds listed in Table 1 are among those for which relatively numerous interactions have been reported; however, the abundance of reports may not necessarily reflect greater drug promiscuity, but perhaps only more extensive study. The relatively greater promiscuity established for verapamil may reflect the double pharmacophore: at one end of the molecule a nitrogen atom is separated from the phenyl ring by a four-carbon chain, and, at the other end another nitrogen is linked to a second ring by a two-carbon chain. Thus, verapamil may be accommodated by a wider array of protein specificity pockets. Note that the common pharmacophore is present in drug antagonists of receptors for both peptides and non-peptides.

Structural requirements strict for agonists and competitive antagonists, liberal for allosteric antagonists: Antagonist drugs more common than agonist drugs

The classes of antagonist drugs comprise an almost limitless variety of chemical species, widely divergent with respect to size, conformation, aromaticity, and charge. Obviously, inhibition of agonist-induced protein conformation may result from any one of multiple modes of interaction between antagonist and amino acid chains of target proteins. In contrast, physicochemical features of agonists required to trigger a specific conformational transition in a protein are much more rigorous; consequently, active analogs are not so commonly encountered. Agonist drugs are characterized, generally, by their smaller mass, the presence of polar substituents on, or in lieu of, the aromatic moiety, and a shorter chain between the aromatic group (or polar moiety in the case of some endogenous agonists) and the nitrogen atom. The additional moieties and greater hydrophobicity both are consistent with the greater affinity usually seen with antagonist, as compared to agonist, compounds.

Binding mode rigorous for agonists and versatile for antagonists

A proton transfer triggered by an agonist interacting with the receptor is suggested as the initial step in receptor activation [for example, see Ref. 11]. Presumably, an antagonist inhibits receptor activation (conformational change) by interfering with agonist binding and/or agonist-triggered proton transfer. For any given functional protein there seems to be but one primary drug/hormone/substrate binding-cavity within the hydrophobic core of the macromolecule. Thus, the complex interactions described for agonists and antagonists at a monomeric receptor or enzyme suggests multiple modes whereby inhibitory (or stimulatory) compounds are accommodated within the cavity. The number of binding-crypt subsites for antagonist drugs would appear to bear a direct relationship to the number of unique chemical ligands, each compound with its own "fingerprint" of interacting surfaces and charges.

Table 1. Affinities of drugs for functional proteins*†

Drug	<0.01 μM	<0.1 μM	<1.0 μM	<10 μM	<100 μM	>100 μM
Chlorpromazine	α_1, H_1, S_2	D_2, M, AEBS	M_1, M_2, σ, NaCH	ST, α_2, β, OR, CaM	NMDA, PDE, N, PKC S_1, CaCh	NKA
Imipramine	ST	H_1, S_2	CaCh, M, M_2, NaCh, N, AEBS	β	NMDA	AC, PKC, GABA-R, MAO
Nicardipine Nitrendipine Flunarizine	CaCh	H_1	M, α	α_1, α_2, BDZ D_2, M_1, PDE, NAT	S_1, S_2, CaM, NaCh, CaATPase, MgATPase	
Quinidine		$P450_2$	$P450_1$, α_1, α_2	M_1, NKA, PEM DT	NaCh	KCh
Verapamil		H_1, CaCh	S, S_1, K/Ca, S_2, α_1	H_2, ST, α_2, M, PCP, D_2, DT, GABA-T	β_2, M_1, NAT	β_1, PKC, NaCh, PDE, NKA, TRH
Lidocaine Procaine Tetracaine			BDZ	N, μ, β, NaCh, CaU, MAO	M_2, M, PCP, α_1	CaATPase, NKA, D_2, TRH, MgATPase, AChE, α, AEBS, PKC
Tamoxifen	AEBS, ER	CaCh	CaM, D_2	CaMgATPase, PDE	PKC, $P450_6$, CPK, M	CaCh
Propranolol	BDZ	AEBS	S_2	β, α_1	GR, NaCh	PDE, β ER, CaM, TRH
Amiloride		IMZ		M, PA, NaCh, MAO (A)	α_1, α_2, MAO (B)	β, PAF, PKC, EGF, NKA, PK, GS, PhK, GSK
Haloperidol	σ, D_2	S_2, D_1, α_1	NaCh, KCh, μ, H_1, M, OR, D	α_2	β, S_1, PDE	MAO, M

* The concentration shown at the top of each column represents the upper limit of the values reported for the proteins listed in the column. References are available from the author.

† Abbreviations:

α	alpha-adrenergic receptor
β	beta-adrenergic receptor
σ	sigma receptor
μ	mu opiate receptor
AChE	acetylcholinesterase
AC	adenyl cyclase
AEBS	anti-estrogen binding site
BDZ	benzodiazepine receptor
CaCh	calcium channel
CaM	calmodulin
CaATPase	Ca^{2+}-ATPase
CaMgATPase	Ca^{2+}, Mg^{2+}-ATPase
CaU	calcium uptake by sarcoplasmic reticulum
CPK	Ca^{2+}-dependent protein kinase
D	dopamine receptor
DT	dopamine transporter
EGF	epidermal growth factor receptor
ER	estrogen receptor
GABA-R	γ-aminobutyric acid receptor
GABA-T	γ-aminobutyric acid transport
GR	glucucorticoid receptor
GS	glycogen synthase
GSK	glycogen synthase kinase
H	histamine receptor
IMZ	imidazoline receptor
K/Ca	K^+-stimulated Ca^{2+} uptake
KCh	K^+ channel
M	muscarinic receptor
MAO	monoamine oxidase
MgATPase	Mg^{2+}-ATPase
N	nicotinic receptor
NaCh	sodium channel
NAT	noradrenaline transport
NKA	Na^+, K^+-ATPase
NMDA	*N*-methyl-D-aspartate-sensitive glutamate receptor
OR	opiate receptor
$P450_1$	11D1
$P450_2$	sparteine oxidation
$P450_6$	ethylmorphine demethylation
PA	plasminogen activator (urokinase type)
PAF	platelet-activating factor receptor
PCP	phencyclidine receptor
PDE	phosphodiesterase
PEM	phosphatidyl ethanolamine methyltransferase
PhK	phosphorylase kinase
PK	protein kinase
PKC	protein kinase C
S	serotonin receptor
ST	serotonin transport
TRH	thyrotropin-releasing hormone receptor

As a consequence, a given labeled drug is most completely and efficiently displaced, commonly, by its unlabeled counterpart relative to other members of its drug class. Binding sites are distinct for either inhibitors or primary ligands, but they may overlap. An antagonist acting allosterically (at binding pocket subsites) may or may not, at the same time, influence the affinity of the natural agonist for binding to the primary recognition site.

The ligand binding domain in the membrane-spanning, hydrophobic core of the β-adrenergic receptor contains a carboxylate group (Asp^{113}) which combines with either agonist or antagonist [2]. Thus, substitution of Asp^{113} inhibits binding of both types of ligand; the binding sites for agonists and antagonists, therefore, must overlap. Substitution of Asp^{79} results in a 10-fold decrease in affinity for agonist but does not change the affinity for antagonists, indicating the differential role of this subsite in aginist and antagonist binding. Additional substitutions within the hydrophobic domain were found to affect binding of agonist, antagonist or both.

Radioligand binding studies define agonist/antagonist interactions that range from mutually potent and competitive, e.g. naloxone and opiate agonists or *d*-tubocuarine and acetylcholine, to cases where agonist and antagonists are only weakly, if at all, mutually inhibitory. Allosteric (non-primary site) influences of antagonist drugs upon binding of agonist (primary site) or other antagonists may be revealed by testing for mutual displacement in binding assays, mutual alteration of rates of association or dissociation, preferential protection against functional group reagents, and preferential modification of either primary or allosteric sites. Only compounds with a serotonin-like structure or some containing the indole moiety compete for labeled 5-HT binding; known antagonists bind weakly or not at all [12]. Conversely, 5-HT has weak (micromolar) affinities for antagonist sites. A given 5-HT antagonist may bind simultaneously to both the 5-HT primary recognition site and an allosteric site, whereas another antagonist may interact only with the allosteric site [13]. Gallamine at 0.01 to 1.0 μM interacts at the acetylcholine binding site on the muscarinic receptor (it competes for binding and protects the site from alkylation by sulfhydryl reagents) [14]. At 10–1000 μM it acts as a non-competitive inhibitor of the receptor, acting at an allosteric site with respect to both acetylcholine and classical muscarinic antagonists such as atropine. Binding of one muscarinic antagonist, quinuclidinyl benzilate (QNB), is stable and of another, pirenzipine, unstable to certain extractant procedures, suggesting different binding modes; however, both antagonist subsites must overlap since QNB competes for [^{3}H]pirenzipine binding [15].

Binding of antagonist drugs to ubiquitous P450 enzymes

Many, if not all, of the drugs that bind to functional proteins also bind to and may be metabolized by members of the family of cytochrome P450 heme proteins (see Table 1). These enzymes have been identified in virtually all cells and tissues and are associated with endoplasmic reticulum, primarily, but to mitochondria, nuclear and other membranes, also. A regulatory site, whose occupation by certain compounds modulates the activity of these enzymes, accommodates a large diversity of drugs [16]. Three major types of drug binding to P450 enzymes have been described, each associated with a particular spectral pattern: type I compounds (e.g. SKF 525A) are substrates that elicit in the heme iron an electronic shift without a direct interaction with the heme; type II compounds interact directly with the heme (ligand binding) but also with the substrate site (imidazoles fall into this class); and reverse type I compounds act at a distinctive site(s) of the substrate-bound form of P450 to displace the substrate [17]. There may be sub-domains within each of these sites to account for distinctive binding modes reported for each of many compounds. There is a great diversity of P450 isozymes that vary widely in their substrate specificities, presumably reflecting differences in amino acid sequences within the binding domains. Major binding proteins in brain for several inhibitors of dopamine transport and in liver for histamine antagonists were identified as a P450 isozyme (desbrisoquine) [18, 19]. Mepyramine is known to be metabolized in liver by P450 enzymes [20]. The binding properties, affinity and drug specificities of a "specific" sigma opiate receptor ligand were identical in membranes from rat brain and in liver microsomes, suggesting that the sigma receptor is a P450 isozyme [21, 22].

Perspectives

The union of a given drug with a particular functional protein is unique; subtle structural variations in the ligand or in the hydrophobic acceptor pocket account for profound differences in both mutual affinity and efficacy. For example, buprenorphine is a partial agonist at mu opiate receptors but a potent antagonist at the kappa receptor subtype [23]. Buspirone is both a potent dopamine antagonist and a partial α-adrenergic agonist in rabbit aorta [24]. Clenbuterol is reported to be an agonist at β_1 and an antagonist at β_2 adrenoceptors [25]. The two-dimensional topographical representation of the common pharmacophore defines, in only a most superficial way, certain molecular features that seem to be necessary for binding within the specificity pocket. It is the conformation of the pharmacophore together with its substituents in three dimensions that affords numerous, subtle variations in binding modes among drugs. This consideration is aptly reflected, for example, in the pharmacological and therapeutic differences between the antipsychotic drug, chlorpromazine, and the antidepressant drug, imipramine, tricyclic compounds whose topographical patterns are, superficially at least, almost identical (Fig. 1). The positions of the two aromatic rings, in the tricyclic compounds can be described by three angles. Nogrady [26] has summarized the conformational differences that arise from replacement in the centre ring of the sulfur with a carbon atom. The substitution affects the binding angle of the ring planes, the annellation angle of the ring axes which pass through carbons 1 and 4 of both aromatic rings, and the

torsion angle of the aromatic rings. In the same vein, geometric isomers of norzimeldine show marked differences in their effects on amine uptake by brain slices [27]. The Z isomer potently inhibits noradrenaline uptake but is a weak inhibitor of 5-HT uptake; the reverse is true for the E isomer. A pair of dihydropyridine enantiomers (optical isomers) that bind with virtually the same affinity to a calcium channel fail to compete with one another for binding and differ in that one is an agonist and the other an antagonist [28]. On the other side of the coin, mutation of a single amino acid residue in the binding domain in the β-adrenergic receptor not only markedly alters ligand specificity, but converts certain antagonist drugs to partial agonists. Similarly, substitution of a single amino acid in a P450 isozyme is associated with a remarkable change in relative affinities among drug substrates [29].

A drug by definition elicits a pharmacological response(s) by inducing conformational changes in proteins or by blocking (or enhancing) those elicited by natural ligands. Conceivably, there may be high-affinity interactions, of certain drugs that possess the common pharmacophore but in binding modes that fail to either elicit or inhibit receptor activation. Different antagonist drugs may bind, in the same or dissimilar modes, but may interact, each in a characteristic spectrum of affinities, with multiple proteins. That a particular antagonist can engage, simultaneously, different subsites within the hydrophobic cavity of a single protein may be misinterpreted in binding analyses as an indication of receptor subtypes. The anxiolytic activity reported for CCK type B receptor antagonists [30] may represent, not necessarily an action mediated through the CCK receptor, but through an interaction with another, yet undefined, protein target.

Virtually all antagonist drugs interact with one or more of the ubiquitous P450 isozymes. Because fatty acid derivatives and steroids are endogenous substrates for the P450 enzymes, drugs may interfere with the generation of functional cellular lipids. The functional significance of high-affinity binding of drugs to the oxygenases may, on the one hand, be minimal and reflect extraneous or trivial drug–protein interactions. On the other hand, the drug–P450 union may in other cases mediate the major pharmacological response [31].

It is unlikely that binding site and subsite dimensions, geometry, charge environments, hydrophobic surfaces and other features will ever be known to the extent that drug design technology will yield a compound with absolute specificity for one species of functional protein. The alternative strategy, screening compounds in multiple protein-specific assays, would appear to be a practical means of estimating the pharmacological profile of any given drug, and could serve to select and, to some extent, design drugs that approach the desired specificity.

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